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Renal brush border membrane adaptation to phosphorus deprivation: Effects of fasting versus low-phosphorus diet

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Renal brush border membrane adaptation to phosphorus deprivation: Effects of fasting versus low-phosphorus diet. Alimentary phosphorus deprivation due to a low-phosphorus diet (LPD) elicits a profound antiphosphaturia and an increase in sodium-dependent inorganic phosphate (Pi) uptake by renal cortical brush border membrane (BBM) vesicles. But, in alimentary phosphorus deprivation due to total fasting, high urinary excretion of Pi persists. In the present study, we determined whether low tubular reabsorption of Pi in fasting is due to a diminished capacity of the specific Pi transport system with the renal cortical luminal BBM or whether it is due to a reduced transepithelial reabsorption of Pi because of metabolic conditions occurring in proximal tubule cells during fasting. Sodium-dependent Pi transport in BBM vesicles isolated from LPD rats was markedly increased compared with fasted rats or rats fed a normal phosphorus diet. Sodium-dependent uptake of D-glucose was significantly lower in LPD rats, compared with fasted animals or animals fed a normal diet. Thus, in contrast to LPD, fasting does not elicit an increase in Pi transport and a decrease in D-glucose transport across the isolated renal BBM. The same differences in BBM transport of Pi were present also in thyroparathyroidectomized rats. Further experiments demonstrated that the adaptation of renal function and the renal BBM transport to LPD are overridden by a subsequent period of total fasting. Results of the present study show that fasting both prevents and reverses the renal response of rats to alimentary phosphorus deprivation. The differences in Pi excretion between fasted rats, LPD rats, and LPD rats subsequently fasted are attributed, at least in part, to specific adaptive changes in sodium-dependent Pi transport across the luminal BBM, rather than to alterations in other cellular (metabolic) components of transepithelial Pi reabsorption in the proximal tubule.

Adaptation de la bordure en brosse rénale à la privation de phosphore: Effets du jeûne comparé à une alimentation pauvre en phosphore. La privation de phosphore alimentaire, obtenue au moyen d'une alimentation pauvre en phosphore (LPD), détermine une chute importante de la phosphaturie et une augmentation de la captation sodium-dépendante de phosphore (Pi) par les vésicules de la membrane de bordure en brosse (BBM). Dans la privation alimentaire de phosphore due au jeûne total, cependant, une excrétion urinaire élevée de phosphore persiste. Dans ce travail nous avons cherché à établir si la faible réabsorption tubulaire de Pi au cours du jeûne est due à une diminution de la capacité du transport spécifique de Pi par BBM ou si elle est liée aux conditions métaboliques prévalant dans les cellules tubulaires proximales du fait du jeûne. Le transport sodium-dépendant de Pi par BBM obtenues de rats LPD est fortement augmenté par comparaison avec les résultats que donnent des rats à jeun ou des rats recevant une alimentation normale en Pi. La captation de D-glucose est significativement plus faible chez les rats LPD par comparaison avec les animaux à jeun ou les animaux recevant une alimentation normale. Ainsi, à l'opposé de LPD, le jeûne ne détermine pas une augmentation du transport de Pi et une diminution du transport du glucose à travers BBM isolées. Les mêmes différences sont observées chez des rats thyroparathyroïdectomisés. D'autres expériences démontrent que l'adaptation de la fonction rénale et du transport par BBM à LPD est effacée par une période ultérieure de jeûne total. Ces résultats montrent que la jeûne empêche et inverse la réponse rénale de rats LPD. La différence d'excrétion de Pi entre les rats à jeun, LPD, LPD ultérieurement à jeun est attribuée, au moins en partie, à des modifications adaptatives spécifiques du transport de Pi sodium-dépendant à travers BBM, plutôt qu'à des modifications d'autres composants cellulaires de la réabsorption transépithéliale de Pi dans le tube proximal.

Both clinical [1] and experimental studies [2, 3] have demonstrated that selective deprivation of dietary phosphorus leads to a marked decrease in the urinary excretion of inorganic phosphate ($U_{Pi}V$) accompanied by increases in the urinary excretion of calcium ($U_{Ca}V$) and magnesium ($U_{Mg}V$). In contrast, total food deprivation, which also curtails alimentary phosphate intake for a comparable period of time, was reported to result in a relatively minor decrease in $U_{Pi}V$ [4–8] with no significant changes in $U_{Ca}V$ and $U_{Mg}V$ [6, 9]. The fractional excretion of phosphate (FE_{Pi}) is considerably reduced in response to a low-phosphorus diet (LPD) [2, 10], but FE_{Pi} was reported to be increased [11] and tubular phosphate reabsorption decreased [5] in short-term fasting compared to normally fed controls. These observations, made on various

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mammalian species, suggest that different renal homeostatic mechanisms are induced by severe limitation in the phosphorus intake by the alimentary tract, depending on whether or not the dietary phosphorus deprivation is a consequence of total starvation.

In identifying the regions of the nephron where the adaptive changes occur, micropuncture [12] and microperfusion [13] studies indicate that increased phosphate reabsorption in the proximal tubule is a major factor contributing to the antiphosphaturic response of the kidney to LPD. Moreover, sodium-dependent transport of phosphate across the isolated brush border membrane (BBM) is markedly enhanced in response to LPD [14–16], suggesting that this initial step in phosphate reabsorption by the proximal tubule is an important renal adaptive change and may well be a factor determining the increased flux of phosphate from the tubular lumen across the tubule wall in response to LPD.

The reason why reduction of the dietary phosphorus intake as a component of total starvation does not lead to an antiphosphaturic response analogous to that elicited by LPD is unresolved. It may be explained, in part, by development of metabolic acidosis during starvation [5], but disturbances in cellular energy metabolism may be more important. There is evidence to suggest that alimentary phosphorus deprivation due to fasting [17–19] stimulates gluconeogenesis in the renal cortex of the rat, a contrasting effect compared with alimentary phosphorus deprivation due to feeding with LPD [20, 21]. Because reabsorption of phosphate across the proximal tubule cell involves phosphate transfer against a concentration gradient, a supply of energy from metabolic processes is required. The importance of a metabolic energy supply for renal transtubular transport of phosphate was demonstrated by the use of metabolic inhibitors in clearance experiments [22] and in studies on the isolated perfused kidney [23] that resulted in a decrease in the maximum phosphate transport rate [22] and an increase in phosphate excretion [23]. This raises the possibility that a disturbance of intermediary metabolism and the consequently reduced energy supply available (as adenosine 5′-triphosphate, for example) for tubular transport processes during total starvation could prevent expression of the avid phosphate reabsorption across the BBM. On the other hand, phosphate uptake across the BBM is driven by an electrochemical gradient and occurs without an immediate supply of energy from intermediary metabolism in the tubule cell. It is possible that the crucial effect of phosphorus deprivation of starvation on tubular reabsorption of phosphate may not be the changes in cellular energy metabolism in proximal tubular cells but, instead, may be a specific decrease in the capacity of the BBM for a sodium-gradient-linked phosphate uptake.

To determine which of these major alternatives (altered BBM uptake or altered intermediary metabolism in tubule cells) determines tubular phosphate reabsorption in fasting, we focused on the analysis of ^{32}P-phosphate uptake by BBM vesicles prepared from rat renal cortex. The isolated BBM vesicle system is unique in the sense that it allows the study of the transport of phosphate (and other substances) across the BBM without the influence of other possible cellular components, namely a metabolic energy supply, that are involved in transepithelial phosphate transfer in the proximal tubules.

Methods

Experimental design. Adult male Sprague-Dawley rats, each weighing 170 to 180 g, were housed individually in metabolic cages and allowed free access to distilled water or tap water (as specified below); the body weight and total urine output were recorded daily. A commercially available low-phosphorus diet (ICN Pharmaceuticals Inc., Cleveland, Ohio) containing 0.07% (weight per weight) phosphorus was used as the low-phosphorus diet (LPD), and the normal-phosphorus diet (NPD) was prepared by supplementing the LPD with a mixture of sodium and potassium phosphates (ratio of monobasic:dibasic salts was 1:4) to a final content of 0.5% (weight per weight) phosphorus, as previously described [15]. Sodium and potassium contents in LPD were supplemented by sodium chloride and potassium chloride. NPD and LPD rats consumed similar amounts of food daily and did not differ in mean body weight at any time in the experiment. Separate groups of animals were placed simultaneously on the different dietary regimens, and throughout the experiment all groups were studied in parallel.

In the first series of experiments (Fig. 1), on each of 6 different days, 15 rats were placed in individual metabolic cages and allowed unlimited access to NPD while they stabilized for days 1 to 3. After day 3, the animals were divided into three groups, 5 rats in each, and the allocation of animals was adjusted so that at this time the groups did not differ in body weight or $U_{Pi}V$. The rats were then deprived of all food or were fed with the specified diets for days 4

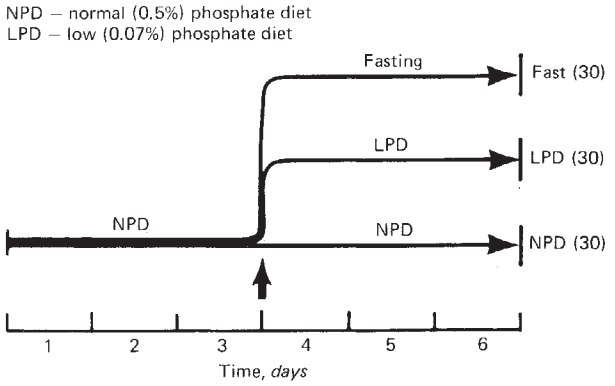

Fig. 1. *Time course of the dietary regimens for the first series of experiments on intact and thyroparathyroidectomized rats.* On each of 6 separate days, 15 rats were placed in individual metabolic cages and stabilized on a normal (0.5%) phosphate diet (NPD) for days 1 to 3. After day 3, the animals were divided into three groups, 5 rats in each, and deprived of all food (fast) or fed with the specified diets (LPD is low-phosphate diet) for days 4 to 6. At the end of day 6, the kidneys were removed from all animals, and in each group of 5 rats the cortices were pooled for the preparation of one brush border membrane fraction as described in Methods. Total number of animals used in each group is given in parentheses.

to 6. The mean body weight between days 3 and 6 (Fig. 1) increased (+22 ± [SEM] 1 g) in the group fed NPD, increased (+18 ± 1 g) in the rats fed LPD, and decreased (−22 ± 2 g) in the fasted group.

An identical procedure was used for rats that had undergone thyroparathyroidectomy (TPTX) 24 hours before the start of day 1 (Fig. 1). A decrease in plasma calcium to 1.85 ± 0.24 mM after TPTX compared with presurgery levels of 2.21 ± 0.13 mM indicated successful TPTX. (Plasma calcium in sham-operated controls was 2.48 ± 0.12 mM compared with 2.24 ± 0.07 mM before surgery). TPTX rats drank tap water supplemented with calcium (90 mg/liter) added as calcium gluceptate (Abbott Laboratories, North Chicago, Illinois).

In the second group of experiments (Fig. 2), 20 rats were housed in individual metabolic cages on each of 4 successive days and stabilized on unlimited NPD for days 1 to 3. After day 3, the rats were divided into two groups (similar body weight and $U_{Pi}V$), 10 rats in each, and were either continued on NPD or fed LPD (Fig. 2). After day 8, both of these groups were subdivided as before into a fed and a fasted group, 5 rats in each. From days 9 to 12 inclusive, the mean body weight increased (+10 ± [SEM] 2 g) in the group fed NPD, increased (+4 ± 1 g) in the LPD group, decreased (−31 ± 3 g) in fasted rats previously fed NPD, and decreased (−31 ± 2 g) in the group fasted after being fed the LPD.

On the last day of all series of experiments, the rats were anesthetized with ethyl ether anesthesia.

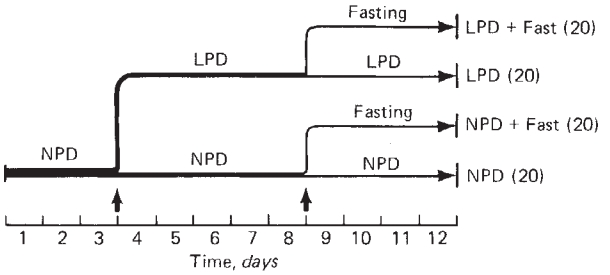

Fig. 2. *Time course of the second set of experiments.* Five rats were placed in metabolic cages on each of 4 successive days and fed NPD. After day 3, the rats were divided into normal (0.5%) phosphate diet (NPD) and low-phosphate diet (LPD) groups, and then, after day 8, each of these was divided into fasted and fed groups. All other conditions were identical to the first experiment. Total number of animals used in each group is given in parentheses.

Venous blood was drawn, and the kidneys were removed and chilled immediately by immersion in an ice-cold solution of 154 mM sodium chloride buffered with 1 mM Tris-Hepes (pH, 7.5). Renal cortical tissue was dissected free from the medulla and used for the preparation of a BBM fraction. The renal cortex from each group compared in the experiment was processed on the same day, and the transport properties of the BBM vesicle fractions from each group were always compared on the same day.

Preparation of a brush border membrane fraction and transport studies. The renal cortex from 5 animals (placed simultaneously on the same dietary regimen) was pooled to provide one BBM preparation, which was sufficient for measurement of both phosphate and D-glucose uptake. When only phosphate uptake was measured, the BBM vesicles were prepared from 1 to 2 animals. BBM fractions from each group of rats in the experiment were prepared on the same day from homogenized renal cortex, as described by Beck and Sacktor for rabbit kidney [24] and as used and described in detail in our previous studies on rats [15, 25]. Previous experiments [25, 26] confirmed that renal cortical BBM from NPD and LPD rats behave similarly in the course of fractionation of the tissue. Verification of the identity and purity of the BBM vesicle fraction used for transport studies was described in detail in our previous communications [15, 25, 26].

The uptake of ^{32}P-phosphate and ^{3}H-D-glucose by isolated BBM vesicles was measured by the Millipore filtration technique of Beck and Sacktor [24] and as used also by Hoffmann, Thees, and Kinne [27] and described in detail in our previous studies [15, 25]. In each BBM preparation, transport of

phosphate and glucose at different times was measured in triplicate, and the mean ± SEM was entered as $N = 1$. Uptake by BBM vesicles from each of the compared groups of rats (see Results) was always measured on the same day to avoid interassay variations. All solutions used for the preparation of BBM fractions and for transport measurements were filtered through a 0.45-μm Millipore filter on the day of use.

Uptake of both ^{32}P-phosphate and ^{3}H-D-glucose in the presence of sodium chloride increased rapidly: phosphate uptake reached a peak within 1 to 2 min; and of D-glucose, within 0.25 to 0.5 min, the so-called "overshoot" [15, 27–29]. Then, the uptake gradually declined to a much lower level at the "equilibrium point" at 120 min (Fig. 3). A time interval of 120 min was chosen for the "equilibrium point," as in our previous study [15], because the equilibration of ^{32}P-phosphate across the BBM is slow relative to D-glucose (Fig. 3) [27]. The initial "overshoot" of phosphate was abolished when 5 mM sodium arsenate, a competitive inhibitor of phosphate transport [27, 29], was added. Replacement of 100 mM sodium chloride by 100 mM potassium chloride in the medium abolished the initial "overshoot" and reduced phosphate uptake at 1 min by more than 90% (Fig. 3). The initial "overshoot" of glucose was eliminated by the addition of 0.5 mM phlorizin, a specific competitive inhibitor of D-glucose transport [28, 29], or by replacement of sodium chloride by potassium chloride (Fig. 3). At 120 min, the uptake of ^{3}H-D-glucose in the presence of sodium chloride was identical with the uptake in the potassium chloride medium, indicating complete equilibration at this time. In the same BBM vesicle preparation, the sodium-dependent uptake of ^{32}P-phosphate at 120 min was about 16% of the uptake at the peak of the "overshoot" and was slightly higher than the uptake at 120 min in the potassium chloride medium, indicating incomplete equilibration. This likely reflects the slow outflux of ^{32}P-phosphate as mentioned above. In a preliminary experiment, complete equilibration was not achieved even after extension of the incubation time up to 360 min. D-Glucose uptake at 120 min was 58 ± 3 pmoles/mg of BBM protein (Fig. 3) whether measured in the presence or absence of a sodium ion gradient. At this "equilibrium point," the concentration of D-glucose inside the vesicles is the same as that outside (50 μM). Thus, the 1 mg of rat BBM protein used in the present study is composed of a total intravesicular space of 1.2 ± 0.1 $\mu\ell$. This volume is in close agreement with those reported by other laboratories from studies on D-glucose uptake by rat [30] and rabbit [31] renal BBM and with studies in this laboratory on L-proline uptake by rat BBM (Kempson and Dousa, unpublished observations).

Based on these preliminary experiments and previous studies [15], the transport of Pi was measured at 15 and 30 sec, time points representing the "uphill" phase of the uptake; at 60 sec, a time point representing maximum or near maximum (peak) of the "overshoot"; and at the "equilibrium" time point at 120 min. Transport of ^{3}H-D-glucose was measured in parallel at the same time intervals. Results from another laboratory [14, 32] show that the time courses of sodium-dependent uptake of phos-

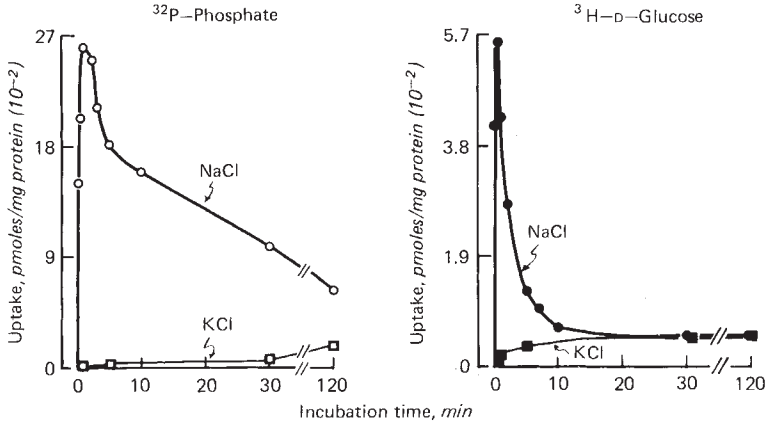

Fig. 3. *Time course of transport by isolated rat renal brush border membrane (BBM) vesicles.* Uptake of ^{32}P-phosphate *(left panel, open symbols)* and ^{3}H-D-glucose *(right panel, closed symbols)* was measured in separate aliquots of the same BBM vesicle preparation. Each point represents the mean of measurements in triplicate from a single BBM preparation from the pooled cortices of five rats. Uptake was determined, as described in Methods, by incubating the vesicles in buffered 100 mM mannitol containing 100 mM sodium chloride (○, ●), or 100 mM potassium chloride (□, ■).

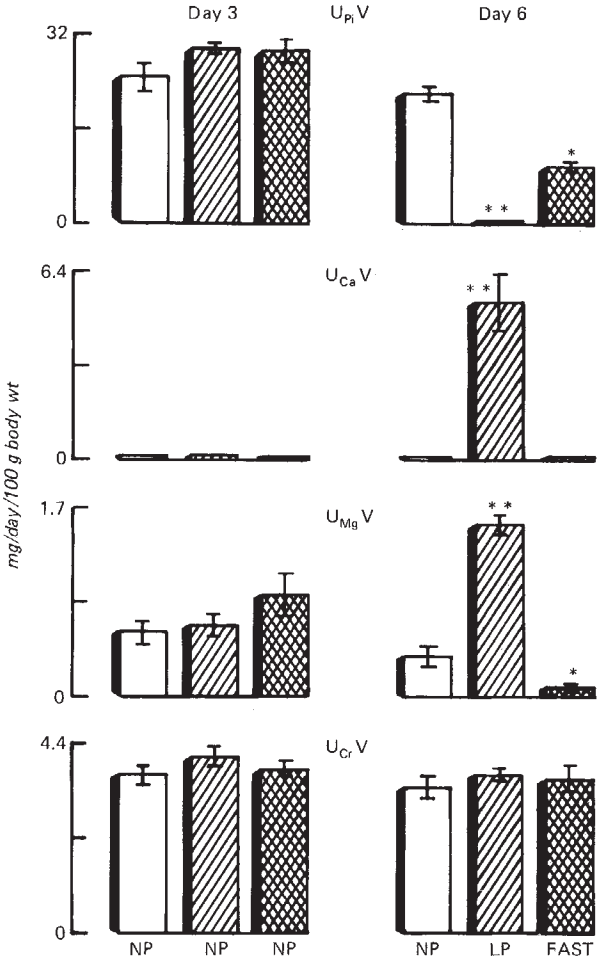

Fig. 4. *Renal adaptation to alimentary phosphorus deprivation due to feeding with low-phosphorus diet and due to total fasting.* Bars represent the mean ± SEM of 30 rats in each group. Cr is creatinine; LP, low-phosphorus diet; NP, normal phosphorus diet; Pi, inorganic phosphate; U_xV = urinary excretion. Day 3 *(left half)* is the last day of the control period (all groups fed NP diet), and day 6 *(right half)* is the last day of the experimental period (groups fed NP diet or LP diet or fasted; see Fig. 1). *One asterisk* (*) indicates significant difference ($P < 0.005$, group t test) compared to the values for NP diet rats on the same day, and *two asterisks* (**) indicate significant differences ($P < 0.001$, group t test) compared to the values for NP diet and fasted rats on the same day.

phate and D-glucose by renal BBM isolated from rats fed diets with different phosphorus contents are similar in the time at which the peak of the "overshoot" occurs.

Analytical methods. Plasma and urinary phosphate concentrations were measured by the method of Chen, Toribara, and Warner [33], and plasma and urinary creatinine concentrations were measured colorimetrically [34]. Calcium and magnesium in plasma and urine samples were determined by an atomic absorption spectrophotometer (Perkin Elmer, model 303). Protein in tissue fractions was determined by the Lowry procedure after solubilization of the samples in 1% sodium lauryl sulphate, as in our previous studies [26, 35, 36]. All analyses were done in duplicates or triplicates. The results were evaluated statistically by Student's t test for paired or group comparisons, as specified under Results. Values of $P > 0.05$ were considered not significant.

Materials. Carrier-free ^{32}P-phosphate (catalog no. NEX-054) was purchased from New England Nuclear (Boston, Massachusetts); and 6-^{3}H-D-glucose, from Amersham Corp. (Arlington Heights, Illinois). All other chemicals and biochemicals were the highest purity grades and purchased from standard suppliers.

Results

By day 6 of the first set of experiments, there were, as expected, striking decreases in $U_{Pi}V$ and increases in $U_{Ca}V$ and $U_{Mg}V$ in LPD rats compared with NPD animals (Fig. 4). Rats deprived of all food for the same time (3 days) excreted a considerable quantity of phosphate, which, although less than that of NPD rats, greatly exceeded that in LPD animals. In the fasted group, $U_{Ca}V$ and $U_{Mg}V$ were much lower than they were in the LPD rats; $U_{Ca}V$ was not different from NPD rats, but $U_{Mg}V$ was reduced to a level significantly lower than that of the NPD group (Fig. 4). The three groups did not differ in plasma creatinine concentrations (Table 1) or in $U_{Cr}V$ (Fig. 4).

Table 1. Effect of 3 days of dietary phosphorus deprivation either by feeding low-phosphorus diet or by total fasting[a]

	NPD rats	LPD rats	Fasted rats
Plasma phosphate, *mM*	2.72 ± 0.06	1.53 ± 0.14[b]	2.72 ± 0.13
Plasma calcium, *mM*	2.36 ± 0.03	2.58 ± 0.05[b]	2.23 ± 0.03[c]
Plasma magnesium, *mM*	0.48 ± 0.01	0.42 ± 0.01[b]	0.58 ± 0.02[c]
Plasma creatinine, *mg/dl*	0.74 ± 0.02	0.78 ± 0.02	0.75 ± 0.01

[a] Values denote the means ± SEM ($N = 30$). LPD is low-phosphorus diet; and NPD, normal phosphorus diet.
[b] Significantly different ($P < 0.001$; group t test) compared to NPD and fasted groups
[c] Significantly different ($P < 0.005$; group t test) compared to NPD rats

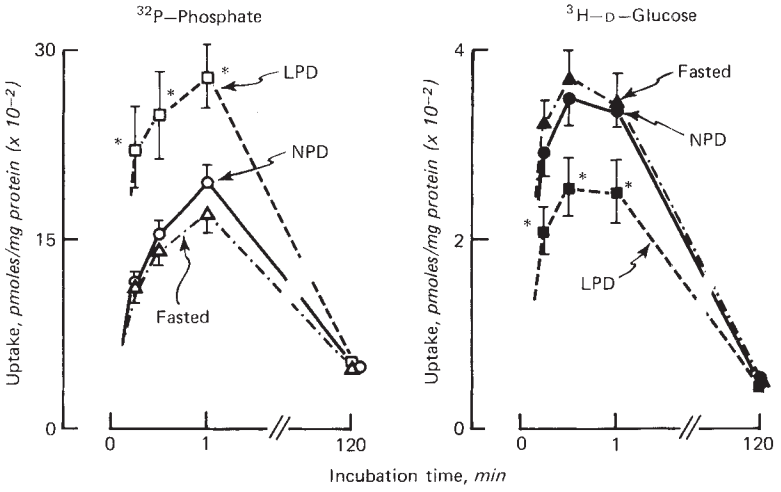

Fig. 5. *Time course of phosphate* (left panel, open symbols) *and* D-*glucose* (right-panel, closed symbols) *uptake by isolated rat renal brush border membrane (BBM) vesicles.* Renal BBM vesicles were prepared at the end of day 6 (Fig. 1) from rats fed normal-phosphorus diet (NPD; —○—○—; —●—●—) or low-phosphorus diet (LPD; ---□---□---; ---■---■---) or from fasted rats (-·-·△-·-·△-·-·; -·-·▲-·-·▲-·-·). Each point represents the mean ± SEM for six separate BBM preparations, and each preparation was analyzed in triplicate. The uptake of phosphate and glucose was determined in separate aliquots of the same BBM vesicle preparation and was measured, as described under Methods, after incubation of BBM vesicles in buffered 100 mM mannitol containing 100 mM sodium chloride. The "overshoot" was eliminated when sodium chloride was replaced by potassium chloride; in the potassium chloride medium the uptake (pmoles/mg protein) by vesicles from NPD rats was 30 ± 9 for phosphate (0.5 min of incubation) and 7 ± 2 for D-glucose (0.25 min incubation). The uptake of either phosphate or D-glucose in the potassium chloride medium was not significantly different between the three groups. An *asterisk* (*) denotes a value significantly different ($P < 0.05$ or higher level of significance; paired t test) from the uptake at the same time interval in BBM preparations from NPD and fasted rats.

In LPD rats, at the end of the experiment (day 6), there was a significant decrease in plasma concentrations of phosphate and magnesium and a significant increase in plasma calcium compared with NPD animals (Table 1). In fasted rats, there was no change in the plasma concentration of phosphate, but, in contrast to LPD rats, plasma magnesium was increased and plasma calcium was decreased compared with NPD controls (Table 1).

The results of studies of phosphate and D-glucose transport by BBM vesicles prepared from each of the three groups are summarized in Fig. 5. After just 3 days of LPD, there was a marked and significant increase in the initial "uphill" phase of sodium-dependent BBM transport of phosphate and a marked decline in the initial phase of sodium-dependent D-glucose transport by BBM compared with NPD rats. In fasted animals, on the other hand, the sodium-dependent BBM uptake of phosphate and D-glucose was not different from NPD rats; if anything, phosphate uptake tended to be lower and D-glucose uptake higher in fasted rats than they were in the NPD group. There were no significant differences between the three groups in either phosphate or D-glucose uptake at 120 min—the "equilibrium point."

Fasted animals showed a significant fall (Table 1) in total plasma calcium concentration (ionized calcium was not measured), and preliminary experiments suggest that plasma levels of parathyroid hormone (PTH) tend to increase in rats fasted for 24 hours (Dr. H. Heath, III, Endocrinology Research Unit, Mayo Clinic; personal communication). In contrast, the plasma calcium concentration was elevated in LPD rats (Table 1), and the serum PTH levels reportedly [37] tend to fall in young rats fed LPD. Because phosphate transport in isolated BBM vesicles was reported to decrease in response to injection of intact rats with PTH [14, 32], additional control experiments were performed to determine whether or not the observed differences in BBM uptake of phosphate might be due to changes in PTH secretion stimulated by the changes in plasma calcium concentration. TPTX animals were treated in a manner similar to that used in the previous experiments (see Methods); and by day 6, values for $U_{Pi}V$ in the three TPTX groups were similar to those in the intact groups (Fig. 4). As summarized in Fig. 6, phosphate transport in BBM preparations from TPTX animals changed in a way similar to that of BBM preparations from the corresponding group of intact rats (Fig. 5). Transport of phosphate in LPD

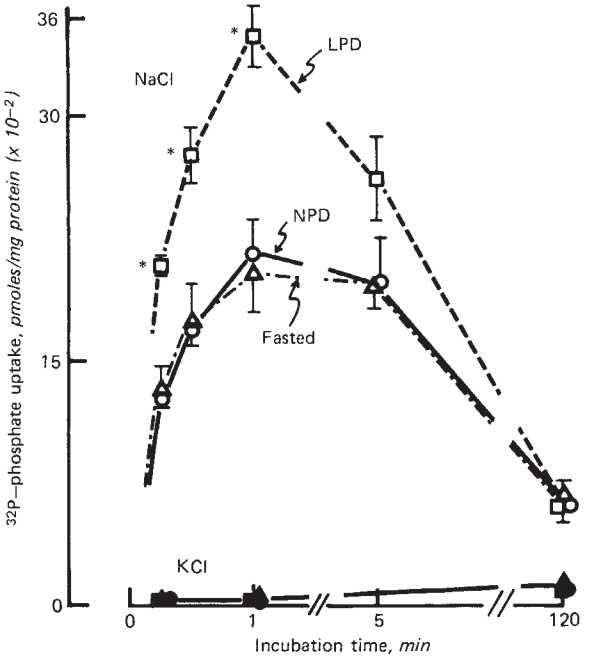

Fig. 6. *Phosphate uptake by renal brush border membrane (BBM) vesicles prepared at the end of day 6 (Fig. 1) from thyroparathyroidectomized rats.* BBM vesicles were isolated from rats fed a normal-phosphorus diet (NPD; —○—○—; —●—●—) or a low-phosphorus diet (LPD; ---□---□---; ---■---■---) or from fasted rats (-·-·△-·-·△-·-·; —▲—▲—). *Open symbols* denote transport in the presence of 100 mM sodium chloride in the incubation medium. *Closed symbols* denote phosphate uptake when sodium chloride was replaced by potassium chloride. Each point represents the mean ± SEM for three separate preparations, and each BBM preparation was analyzed in triplicate. An *asterisk* (*) denotes values significantly different ($P < 0.025$, or higher level of significance; group t test) from the uptake at the same time interval in NPD and fasted rats. Details are given in Methods.

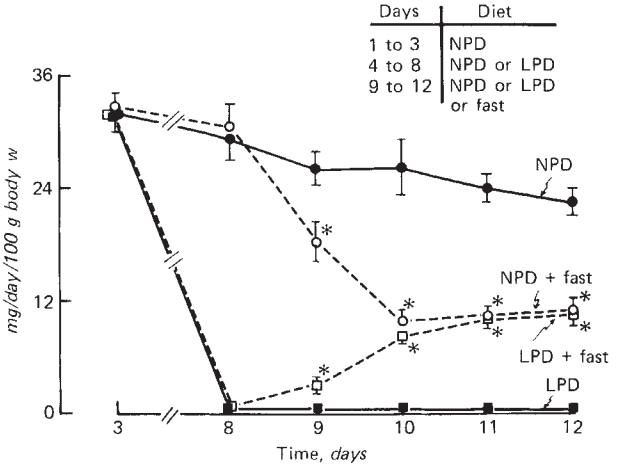

Fig. 7. *Urinary excretion of phosphate when rats fed normal- (NPD) or low-phosphate diet (LPD) were subsequently fasted for 4 days beginning after day 8 (Fig. 2).* Urine was collected every 24 hours from fasted NPD (---○---○---) and fasted LPD (---□---□---) rats. At the same time, urine was collected from other rats fed continuously with NPD (—●—●—) or LPD (—■—■—). Bars represent the means ± SEM of 20 rats in each group. An *asterisk* (*) indicates significant differences ($P < 0.02$; group t test) compared with the values for NPD and LPD rats on the same day. Urinary excretion of calcium and magnesium by these animals is given in Fig. 8.

animals was increased significantly in the initial "uphill" phase compared with NPD groups, but in the rats fasted completely, the phosphate uptake was not different from NPD rats. Uptake at the "equilibrium point" (120 min), or when sodium chloride was replaced by potassium chloride, was not significantly different between the three groups.

The effect of subsequent fasting on BBM phosphate transport and other renal functions in NPD and LPD rats was examined in the second series of experiments (Fig. 2). The groups that were fed continuously for days 9 to 12 maintained the urinary excretions of phosphate, calcium, and magnesium present at day 8 (Figs. 7 and 8); but, in contrast, fasting led to striking changes in urinary ion excretion within 24 hours. In fasted rats previously fed LPD, the $U_{Pi}V$ increased and stabilized by day 12 at a level about 35-fold higher compared with LPD rats (Fig. 7); this occurred without a change in plasma phosphate concentration (Table 2). In the same animals, the hypercalciuria and hypermagnesiuria due to LPD were completely reversed by fasting (Fig. 8). In fact, $U_{Ca}V$ was reduced to an amount slightly but significantly lower compared with NPD rats (upper panel, inset), and $U_{Mg}V$ showed a similar trend (lower panel). Although plasma calcium concentration fell in the fasted LPD group, there was a rise in plasma magnesium concentration (Table 2) compared with the LPD group. In NPD rats, as before (Fig. 4), fasting led to a decrease by about 49% in $U_{Pi}V$, a small but significant decrease in $U_{Ca}V$, and a similar trend in $U_{Mg}V$, although the differences were not significant compared to NPD; $U_{Pi}V$ and $U_{Ca}V$ fell to levels similar to those in fasted LPD rats (Figs. 7 and 8). As before (Table 1), plasma calcium was lower, and plasma magnesium was higher in the fasted NPD animals, compared with the NPD group (Table 2); plasma phosphate in these experiments, perhaps due to the slightly longer period of fasting, was lower in the fasted NPD group compared with the fed NPD rats (Table 2). There were no differences in plasma creatinine concentrations between the four groups (Table 2).

As expected, after 9 days of LPD, there was a marked and significant increase both in the initial "uphill" phase (0.5 min) and at the peak (1.0 min) of sodium-dependent BBM transport of phosphate (Fig. 9). In LPD rats, in this series of experiments,

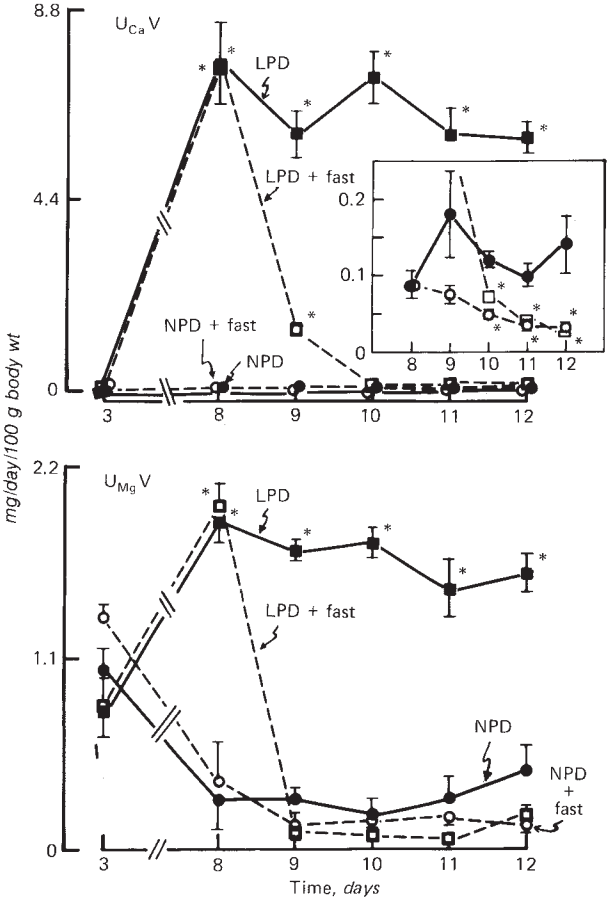

Fig. 8. *Daily urinary excretion of calcium ($U_{Ca}V$;* upper panel) *and magnesium ($U_{Mg}V$;* lower panel) *when rats fed normal-(NPD) or low-phosphate diet (LPD) were subsequently fasted for 4 days.* Inset *(upper panel):* the scale of the abscissa has been expanded to show in detail the calcium excretion on days 9 to 12 by NPD (—●—●—), fasted NPD (---○---○---), and fasted LPD (---□---□---) rats. An *asterisk* (*) indicates significant difference ($P < 0.005$; group *t* test) compared with the values for NPD rats on the same day. See legend to Fig. 7 for other details.

sodium-dependent uptake of D-glucose at 0.25 and 0.5 min was lower compared with NPD rats but did not reach statistical significance. In both fasted groups, regardless of whether fed previously with NPD or LPD, sodium-dependent BBM uptake of phosphate and D-glucose was not significantly different from that of NPD rats. As a distinct trend, phosphate uptake tended to be lower, and D-glucose uptake higher in fasted rats compared with the NPD group. There were no significant differences between the four groups in either phosphate or D-glucose uptake at 120 min—the "equilibrium point."

Discussion

Our observations show that the intrinsic properties of the brush border membrane (BBM) transport systems for phosphate and for D-glucose differ during the phosphorus deprivation of fasting compared to the phosphorus deprivation of a low-phosphorus diet (LPD). Transport across the luminal BBM, the initial step in tubular reabsorption, may be a determining factor (irrespective of metabolic changes) accounting for differences in the renal handling of phosphate and D-glucose in these two states of alimentary phosphorus deprivation. After feeding rats with LPD for 3 days, the sodium-dependent BBM uptake of phosphate in the "uphill" phase was markedly increased (Fig. 5). But, the adaptive response of the BBM phosphate transport system to phosphorus deprivation as a component of total food deprivation for 3 days was different from the response to selective phosphorus deprivation of feeding LPD: phosphate uptake by BBM vesicles from fasted rats was significantly lower at 0.25, 0.5, and 1.0 min compared with LPD rats. Further, the adaptive response of the phosphate transport system in BBM from LPD rats is overridden and completely reversed by the response to a subsequent 4-day fast.

The absence of an increase in sodium-dependent phosphate uptake by BBM from fasted rats and the reversal by subsequent fasting of the effects of LPD on phosphate transport are unlikely to be due to a defect in BBM sodium transport because renal conservation of sodium occurs in the fasted rat [7] unlike the natriuresis observed during fasting in the rabbit [38, 39] and man [4, 40]. More importantly, comparison with another sodium-dependent BBM

Table 2. Effect of 4 days of total fasting on rats previously fed either normal or low-phosphate diet[a]

	NPD rats not fasted	rats fed NPD then fasted	LPD rats not fasted	rats fed LPD then fasted
Plasma phosphate, *mM*	2.42 ± 0.11	2.08 ± 0.06[b]	2.07 ± 0.13[b]	2.15 ± 0.09
Plasma calcium, *mM*	2.66 ± 0.07	2.37 ± 0.04[b]	2.79 ± 0.04[b]	2.28 ± 0.04[c]
Plasma magnesium, *mM*	0.58 ± 0.02	0.74 ± 0.02[b]	0.41 ± 0.01[b]	0.64 ± 0.02[c]
Plasma creatinine, *mg/dl*	0.60 ± 0.03	0.58 ± 0.03	0.58 ± 0.02	0.53 ± 0.04

[a] Values denote the means ± SEM ($N = 20$). LPD is low-phosphorus diet; NPD, normal phosphorus diet.
[b] Significantly different ($P < 0.050$; *t* test) from NPD rats
[c] Significantly different ($P < 0.001$; *t* test from LPD rats

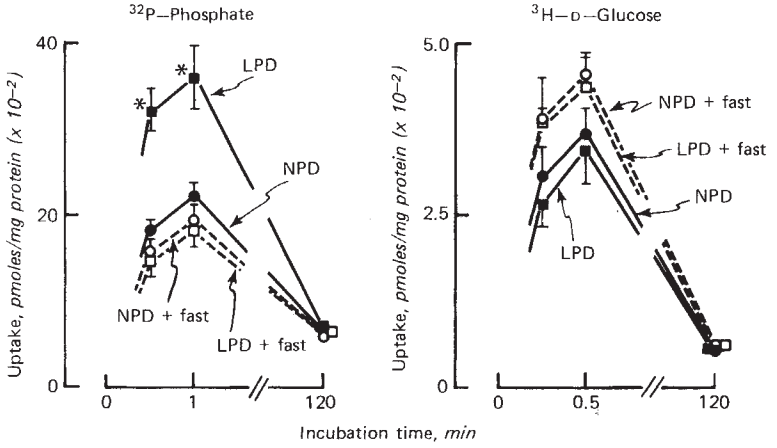

Fig. 9. *Time course of phosphate* (left panel) and *D-glucose* (right panel) *uptake by isolated brush border membrane (BBM) vesicles.* The vesicles were prepared at the end of day 12 (Fig. 2) from rats fed normal-phosphorus diet (NPD; —●—●—), from rats fed low-phosphorus diet (LPD; —■—■—), from fasted NPD rats (---○---○---), and from fasted LPD rats (---□---□---). Each point represents the mean ± SEM for 3 separate BBM preparations from each group. Uptake shown is that measured in the presence of 100 mM sodium chloride; when sodium chloride was replaced by potassium chloride the uptake of phosphate, and D-glucose was not significantly different between groups. An *asterisk* denotes a value significantly different ($P < 0.05$ or higher level of significance; paired t test) from the uptake at the same time interval in BBM from the other groups. See legend to Fig. 5 for other details.

transport system (D-glucose) also argues against such a possibility: sodium-dependent uptake of D-glucose was not decreased, but tended to be higher, in fasted rats. After 3 days on LPD, sodium-dependent D-glucose uptake measured at 0.25, 0.5, and 1.0 min ("uphill" phase) was significantly decreased by 28%, 27%, and 26% (mean values), respectively, compared with NPD rats. A similar but less marked trend was present in the second group of experiments (Fig. 9) in which rats were fed LPD for a longer period, a total of 9 days (Fig. 2). These findings are in agreement with the distinct trend observed in our previous studies [15, 25] when a decrease in D-glucose uptake at the same time intervals accompanied the increase in phosphate uptake in LPD rats. According to recent reports from other laboratories, sugar transport by renal BBM vesicles in LPD rabbits [16] and LPD rats [14, 32] was not significantly different from NPD controls. It should be noted that in the experiments by Stoll et al [14, 32], rats were fed LPD for 7 days or more. Because we observed a marked decrease in D-glucose uptake by BBM vesicles from animals fed LPD for only 3 days (Fig. 5) but little difference after 9 days of LPD (Fig. 9), such a comparison may suggest that decreased uptake of D-glucose by BBM vesicles occurs only in the early phases of adaptation to LPD. As in earlier studies from this laboratory [15, 25], uptake by BBM vesicles was measured at a pH of 8.5 to ensure optimal conditions for phosphate transport. Previous work has shown that sodium-dependent phosphate uptake is increased at more alkaline pH [16, 27] whereas sodium-dependent D-glucose uptake is not altered significantly by similar pH changes (Kempson and Dousa, unpublished results).

The reciprocal changes during fasting in two sodium-dependent transport processes in the BBM underline their specificity, but the stimuli which elicit these changes are not yet identified. The changes are not due to competition for the electrochemical driving force because phosphate uptake and D-glucose uptake were measured in the absence of other transported solutes. It is of interest to note that a decrease in the maximum glucose transport rate in response to selective deprivation of dietary phosphorus was observed in clearance experiments on dogs [41]. A decrease in the sodium-dependent D-glucose transport across the BBM observed in the present study (Fig. 4) may be the cellular basis for the decreased capacity of the proximal tubule to reabsorb glucose in LPD animals.

The adaptation of phosphate transport in response to LPD persists in the isolated perfused kidney [42], isolated microperfused tubules [13], and isolated BBM [14–16]. Such considerations are compatible with a possibility that BBM adaptive changes in response to LPD and fasting are elicited by factor(s) that may be of a humoral nature. Injection of rats [14, 43] and dogs [44] with PTH caused a small decrease in phosphate uptake by the isolated BBM, and there have been recent reports from this [45] and other [14, 32] laboratories of a slightly higher phosphate uptake by renal BBM from chronically

TPTX or parathyroidectomized rats compared to sham-operated rats. It was necessary, therefore, to examine the possibility that the absence of increased BBM uptake of phosphate in fasted animals might have been due to an increase in plasma levels of PTH during fasting. Increased plasma PTH levels during short-term starvation may be induced by the fall in plasma calcium observed in the fasted group (Table 1). The results of the experiment on TPTX animals (Fig. 6) demonstrate that the differential BBM adaptation to phosphate deprivation caused by feeding LPD or by fasting occurs in the absence of endogenous PTH, calcitonin, and thyroid hormones, thus excluding these hormones from a possible role in the increased phosphate uptake in LPD (in agreement with recent reports [14, 32]) and, more specifically, in the lack of such an increase in phosphate uptake during fasting.

The observed changes in $U_{Pi}V$ during 72 hours feeding with LPD or fasting (Fig. 4) are in basic agreement with the results of studies reported on the rat [5, 7] and hamster [6]. In addition to the changes in $U_{Pi}V$, we reported two new features of renal electrolyte handling associated with total fasting compared with selective dietary phosphate deprivation. These are the absence of hypercalciuria and, in spite of an elevated plasma magnesium concentration (Table 1), a lower $U_{Mg}V$. The latter observation suggests increased renal tubular reabsorption of Mg in fasting. Because in the fasted group $U_{Pi}V$ was higher and $U_{Ca}V$ and $U_{Mg}V$ were both lower compared with LPD rats, changes similar to a preliminary report on the hamster [6], such comparisons indicate that higher $U_{Pi}V$ is not a result of a generalized decrease in reabsorption of solute by the proximal tubule during the short-term fasting. Higher $U_{Pi}V$ in fasted rats also is not explained solely by the higher plasma phosphate concentration in fasted rats compared with the LPD group (Table 1) because plasma phosphate concentration was similar in fasted NPD rats and fed LPD rats but there was still a marked difference in $U_{Pi}V$ between these two groups (Table 2 and Fig. 7). Moreover, in a recent study [5], the filtered load of phosphate was found to be decreased in rats during fasting. The lower plasma phosphate in fed LPD rats, compared with fed NPD rats, in both experiments (Tables 1 and 2) is not the sole cause of the low $U_{Pi}V$ in the LPD group for the following reasons: *First,* renal adaptation to LPD is not always accompanied by a fall in plasma phosphate [25, 26]. *Second,* it was shown that when hypophosphatemia does occur in LPD rats fractional reabsorption of phosphate is always higher compared with NPD animals, even when plasma phosphate in both groups is increased to almost 6 mM by phosphate infusion [2].

The changes in $U_{Pi}V$ and $U_{Ca}V$ when LPD rats are subsequently fasted are in a direction similar to those in LPD hamsters which were reported recently in preliminary form [46]. Unlike the LPD hamster fasted for 16 hours, LPD rats showed no change in plasma phosphate after 4 days of fasting. In the fasted LPD rats, a decrease in $U_{Mg}V$ occurred despite a rise in plasma magnesium, again suggesting that there may be increased tubular reabsorption of magnesium during fasting. The reciprocal changes in $U_{Pi}V$ and $U_{Mg}V$ (Figs. 7 and 8) indicate that the reversal by fasting of the antiphosphaturic effect of feeding LPD is not due to a generalized decrease in tubular reabsorption. Further, this reversal is unlikely to be accounted for by a major increase in GFR because plasma creatinine did not differ. The effects of fasting on $U_{Ca}V$ and $U_{Mg}V$ in NPD rats are difficult to evaluate because of the already low excretion rates in fed NPD rats compared with fed LPD rats. On the basis of the urinary excretion data, it appears that fasted rats reach the same steady state, irrespective of their previous dietary phosphate intake.

Conclusion. The response of the kidney to dietary phosphorus deprivation, in terms of antiphosphaturia and a specific increase in sodium-dependent phosphate transport across the BBM, does not occur during total deprivation of all food. The present results suggest that the continuously higher $U_{Pi}V$ during the phosphorus deprivation of fasting (compared to LPD) arises because the capacity for sodium-dependent phosphate transport across the renal BBM remains relatively low in this pathophysiologic situation. The lack of BBM adaptation to alimentary phosphorus deprivation, rather than changes in cellular metabolism, may be the sole or contributing cause of persistent renal phosphate excretion during starvation. Rapid reversal by total fasting of the antiphosphaturic effect of LPD is likely to be determined by a decrease in the capacity for sodium-dependent phosphate uptake across the luminal BBM of proximal tubular cells. Moreover, subsequent fasting reverses also the hypercalciuric and hypermagnesiuric effects of LPD.

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